Distribution of Tryptophan Hydroxylase Immunoreactivity in the Spiral Ganglion Neurons of Mouse Cochlea

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Abstract Serotonin, also known as 5-hydroxytryptamine, is an important neurotransmitter and neuromodulator in the nervous system. Spiral ganglion neurons (SGNs) are the first station of the peripheral afferent auditory nervous system. We have previously demonstrated positive serotonin immunoreactivity in mouse SGNs. Whether serotonin in the SGNs is absorbed from the circumstances, or is synthesized by the neurons themselves is unknown. The purpose of this study was to find out the source of serotonin in the SGNs. Tryptophan hydroxylase is the rate-limiting enzyme in the process of serotonin biosynthesis. We detected tryptophan hydroxylase in the mouse SGNs using immunohistochemistry. The positive immunostaining of tryptophan hydroxylase was observed in the cytoplasm of

SGNs. The cytoplasm of the SGNs was not get stained in the negative control group. Our results suggest that SGNs can synthesize serotonin themselves, and they may serve as an additional source of serotonin in the cochlea. The SGNs are serotonergic neurons. The receptors of serotonin on the membrane of SGNs interact with serotonin as auto-receptors. Serotonin in these neurons plays its physiological role in an autocrine/paracrine way.

Keywords Tryptophan hydroxylase · Serotonin · Spiral ganglion neuron · Immunohistochemistry

Abbreviations

5-HT 5-Hydroxytryptamine
SGN Spiral ganglion neuron
TPH Tryptophan hydroxylase

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Introduction

Serotonin, also known as 5-hydroxytryptamine (5-HT), was isolated in 1948 [1] and is widely distributed in different species ranging from insects to humans. In addition to its role in vasoconstriction, serotonin may also act as a neuromodulator/neurotransmitter [2].

Serotonin and its receptors are widely existed, including in the mammalian cochlea. However, the source and role of serotonin in the cochlea remain poorly understood. Two sources of serotonin in the cochlea, serotonergic fibers and the blood, have been reported [3, 4]. Serotonergic fibers are found to project to the area below the hair cells of cochlea. These fibers are originated from brainstem neurons. The function of these fibers has been suggested to be involved in

the modulation of the ascending afferent system, but not the transduction of sound during auditory process. It was proposed that other than the sound transduction process, the serotonergic fibers might be involved in modulation of sleep and awake, state of urgency, attention and alertness [3, 5].

Another source of serotonin in the cochlea is the blood. The concentration of serotonin in the cochlea was reduced by about 76% after removing the blood by aortic perfusion [4]. Serotonin in the blood can induce a reduction of blood flow in cochlea, which can result in dysfunction of the cochlea microcirculation [6].

In our previous research, positive immunoreactivity of serotonin was detected in the spiral ganglion neurons (SGNs) of adult mouse [7]. In the retina, serotonin can be synthesized by one type of amacrine neuron, other than the serotonergic fibers originating from the brainstem raphe nuclei [8]. This is one case of serotonergic synthesis by peripheral nervous system, instead of central nervous system. SGNs are the first station of the afferent auditory nervous system. Can serotonin in SGNs also be synthesized by the SGNs of cochlea or is it released from the surrounding tissues? Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the process of serotonin biosynthesis. To address whether SGNs can serve as an additional source of serotonin in the cochlea, we examined the expression of TPH in the mouse SGNs by immunohistochemistry.

Materials and Methods

Kunming mice of both sexes at about day 30 were used for immunohistochemical analysis. In the experiment, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice in which the studies were conducted. The TPH polyclonal antibody was obtained from Abcam (Cambridge, MA, USA). The HistostainTM Plus Kits and Haematoxylin were purchased from Zhong Shan Golden Bridge Biology Technique Corporation (Beijing, China). The 3, 3-Diaminobenzidine solution was purchased from Sigma (St Louis, MO, USA).

Kunming mice were divided into study group and control group. The experiment process of immunohistochemistry was previously described by Long et al. (2008). In the study group, Nembutal (50 mg/kg, i.p.) was used to make the mice deeply anesthetized. The mice were then decapitated. The mice cochlea tissue was fixed with 10% paraformaldehyde at room temperature for 48 h. The tissue was embedded in paraffin and serially sectioned after decalcification and dehydration. After the tissue sections

been deparaffinized and rehydrated, the antigen was retrieved by 3% hydrogen peroxidase for 10 min. Non-specific binding sites were blocked by 5% normal rabbit serum. The rabbit anti-TPH antibody (diluted to 1:200) was incubated with the sections for 60 min at 37 °C. Biotin-labeled secondary antiserum was subsequently applied for 15 min at 37 °C. Streptavidin–biotin–peroxidase complex was then added at 37 °C for 15 min. After each step, except after the application of the blocking solution, the tissue sections were rinsed with 0.01 M phosphate-buffered saline (pH 7.4) 3 times. The sections were stained with 3, 3-Diaminobenzidine solution, and then counterstained with haematoxylin. The tissue sections were examined with an Olympus light microscope.

In the control group, the same protocol was used, except that the primary antibody was replaced by phosphate-buffered saline.

Results

The SGNs in the center of the photogram were larger than surrounding cartilage cells (Figs. 1, 2). In the study group, TPH-positive immunoreactivity was observed in the cytoplasm of SGNs (Fig. 1). In the negative control group, the cytoplasm of the SGNs was not stained (Fig. 2).

Discussion

Previous report from our group showed positive immunoreactivity for serotonin in these neurons [7], suggesting that serotonin may be synthesized in the SGNs and

Fig. 1 The SGNs in the adult mouse cochlea were larger than surrounding cartilage cells and positively stained with anti-TPH antibody in the cytoplasm ($\times 200$). The arrow indicates one of the SGNs

Fig. 2 No positive immunoreactivity in the cytoplasm of the SGNs ($\times 400$) in the negative control group (without the primary antibody of TPH)

act as a neurotransmitter/neuromodulator in these cells. TPH is a rate-limiting enzyme in the process of serotonin biosynthesis. In the present study, we reported that TPH was expressed in the SGNs of mouse cochlea. Besides the serotonergic fibers which project to the inner and outer hair cells, and the blood, the SGNs are proven to be the third source of serotonin in the cochlea. Majority of the serotonin in human body is present in enterochromaffin cells in the gut, where it regulates intestinal movements [2]. The remainder is synthesized by serotonergic neurons which are located in the central nervous system [2]. SGNs belong to the peripheral nervous system. Detection of serotonin and TPH in the SGNs helps to complement a whole knowledge of serotonergic innervation in the mammalian. There are two forms of TPH: TPH1, found in several tissues, and TPH2, a brain-specific isoform [9]. The antibody used in our experiment was not isoform-specific. Detection of TPH2 in the SGNs is needed in the future to further confirm the existence of serotonin biosynthesis in the peripheral nervous system.

Cochlear nuclei and the inferior colliculus serve as relay stations for the process of auditory information in the central nervous system. Previous studies have shown that serotonin can modulate the auditory process in the dorsal cochlear nucleus [10]. It may change tone-evoked responses in many inferior colliculus neurons [11]. These are evidences of serotonergic innervation of the auditory afferent pathway in the central nervous system. However, the role of serotonin in the peripheral auditory nervous system was nearly unknown. The results of the present study suggest that serotonin can be synthesized by the SGNs. The SGNs are serotonergic neurons. The receptors of serotonin on the SGNs may act as autoreceptors. The interaction of serotonin with these receptors may be in an autocrine/paracrine manner.

In conclusion, in this study, we found that TPH, the rate-limiting enzyme in the process of serotonin biosynthesis was positively immunostained in the cytoplasm of mouse SGNs using immunohistochemistry. The result is an evidence of serotonin synthesis by the SGNs. The SGNs in the cochlea are the primary neurons of the auditory afferent system. Serotonin may play an important role in the auditory transmission.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice in which the studies were conducted.

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